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Letter to the Editor

Reliability of M protein quantification: comparison of two peak integration methods on Capillarys 2

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In the clinical follow-up of patients with monoclonal gammopathies, the concentration of the monoclonal immunoglobulin (M protein) is commonly determined by serum protein electrophoresis and integration of the M protein peak in the electropherogram (1–3). Integration is usually performed by the perpendicular drop method which includes monoclonal as well as background polyclonal immunoglobulins (Figure 1A). Previous studies by Bergón et al. (4) showed that these methods tend to overestimate M proteins at low concentrations and that polyclonal background may be the cause of this effect. Alternatively, integration by tangent skimming may be used (Figure 1B).

In this study, the two methods of integration were compared using a Capillarys 2 capillary zone electrophoresis system (Sebia, Issy-les-Moulinaux, France). The linearity of M protein determination was assessed by dilution series (Figure 2). In agreement with previous findings (4), the perpendicular drop method tended to overestimate M protein concentrations below 15 g/L. In contrast, the tangent skimming method showed no significant deviation from the calculated values down to 1.5 g/L for M proteins of both the IgA and IgG type. Contrary to the findings of Bergón et al. (4), that subtraction of calculated polyclonal γ-globulins resulted in too low M protein concentrations, no underestimation of M protein concentrations was observed with the tangent skimming method.

To further evaluate the effect of the integration method, M proteins of the IgA, IgG, and IgM type were determined in parallel by the perpendicular drop method and by tangent skimming. Linear regression analysis according to Passing and Bablok (5) showed good correlation between both integration methods with a bias for the perpendicular drop method of 2.6 g/L at the y-axis intercept ($M\ protein_{perp.\ drop} = 0.979 \times M\ protein_{tangent} + 2.59$; $r = 0.997$). The perpendicular drop method yielded higher values at all M protein concentrations (Figure 3, inset). However, this difference contributed most to the overestimation of M proteins at the lowest M protein concentrations, although discrepancies of up to 58% were already observed for some sera in the concentration range of 10–20 g/L (Figure 3). Differences between the two methods were highest in sera containing high background concentrations of polyclonal immunoglobulins. Table 1 illustrates the effect of background immunoglobulin concentration in the case of a patient

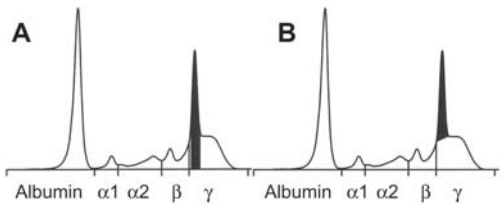

Figure 1 Methods of peak integration.
(A) Perpendicular drop method and (B) tangent skimming method.

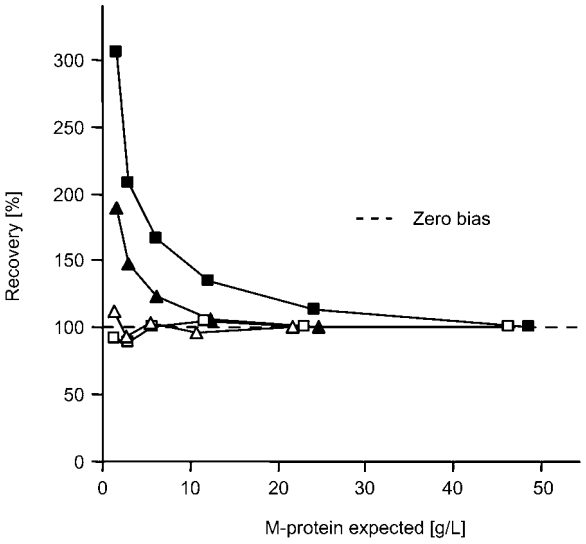

Figure 2 Linearity of M protein quantitation assay using different integration methods.
Serum containing 50 g/L monoclonal IgG (squares) or 25 g/L monoclonal IgA (triangles) was diluted in steps of 1:2 with normal serum containing 12.3 g/L polyclonal γ-globulins and subjected to capillary zone electrophoresis. M protein peaks were integrated either by the perpendicular drop method (closed symbols) or by the tangent skimming method (open symbols). Absolute concentrations of M protein were calculated from the percentage of the area under the curve attributed to the M protein and the total protein concentration as determined by the biuret method on a Hitachi 917 clinical-chemical analyzer (Roche Diagnostics, Rotkreuz, Switzerland). All experiments were carried out in quadruplicates.

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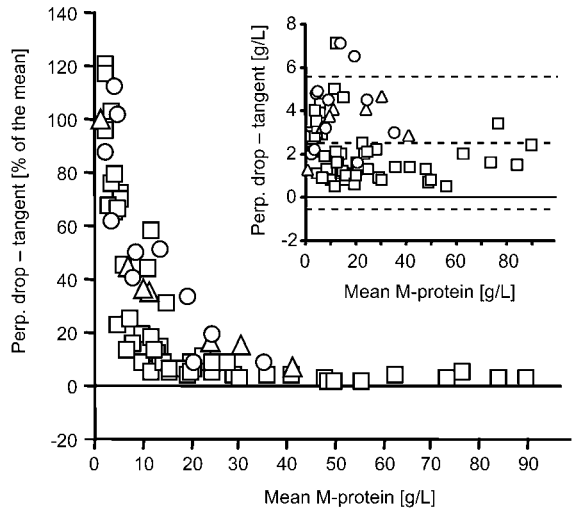

Figure 3 Agreement of M protein quantitation by the perpendicular drop and the tangent skimming method.
A total of 71 samples from patients with monoclonal gammopathies of the IgG (□) IgA (△) or IgM (○) type were analyzed in parallel by the perpendicular drop and the tangent skimming methods, respectively. Differences between the two methods relative to the mean concentration are plotted against the mean concentration of the two methods (6). The inset shows a Bland-Altman plot (7) of absolute differences vs. the mean concentration. The solid line indicates the line of identity, the dashed lines represent the mean difference ±2 standard deviations.

Table 1 Follow-up of a patient with an IgM κ monoclonal gammopathy. Summary of data before and after an increase in polyclonal IgG.

Analyte	Before polyclonal increase, g/L	After polyclonal increase, g/L	Change
Total IgG (nephelometry)	10.2	20.1	+97%
Total IgM (nephelometry)	17.1	17.4	+2%
M protein (perpendicular drop method)	13.4	19.5	+46%
M protein (tangent skimming method)	10.2	10.9	+7%

with Waldenstöm's macroglobulinemia. This patient showed constant concentrations of IgM, but a doubling of polyclonal IgG as determined by nephelometry (BN II, Siemens, Tarrytown, NY, USA) within 6 months. In agreement with the nephelometric assay, quantification of the M protein by the tangent skimming method indicated little change in the M protein concentration in the same period, whereas quantitation by the perpendicular drop method suggested an increase in the M protein concentration of almost 50%. An increase in M protein of more than 25% is one of the criteria to define progression of Waldenstöm's macroglobulinemia (2). Therefore, the accurate quantitation by tangent skimming is critical for the clinical evaluation.

Quantitation of serum proteins by the perpendicular drop method is the common procedure for reasonably separated peaks, such as the albumin, α-, β- and γ-globulin fractions of electropherograms, as well as for M protein peaks with low polyclonal background, which is often the case in malignant gammopathies. However, this study and a previous study (4) show that the perpendicular drop method overestimates M proteins at concentrations below 10–20 g/L, mainly due to background interference. As shown in the present study, M protein peak integration by tangent skimming avoids these limitations. This is of particular importance for the follow-up of patients with monoclonal gammopathy of undetermined significance, which often have normal concentrations of polyclonal immunoglobulins (8) and where increasing M protein levels (9) and M protein levels >15 g/L (10) are major predictors of progression to multiple myeloma or related malignancy. We therefore recommend that the tangent skimming method is used for integration of M proteins.

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